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Lighting-up Protein-Ligand Interactions with Fluorescent PET (Photoinduced Electron Transfer) Sensor Designs[‡]

Bernard McLaughlin,^a Esther M. Surender,^a Glenn D. Wright,^a Brian Daly^a and A. Prasanna de Silva^a

THE FLUORESCENCE OF SENSORS **1** AND **6**, WHICH ARE DESIGNED IN TERMS OF PHOTOINDUCED ELECTRON TRANSFER (PET), ARE SWITCHED 'ON' BY FACTORS OF 15 AND 6 WITH AVIDIN IN A RATHER SELECTIVE MANNER. **3** AND **7**, SIMPLER DERIVATIVES OF **1**, ARE SWITCHED 'ON' BY FACTORS OF 16 AND 7.5 WITH BOVINE SERUM ALBUMIN, THOUGH AT 30-FOLD HIGHER CONCENTRATIONS.

The fluorescent PET sensing/switching design¹ is a useful tool for over 350 laboratories.² The major class of sensors has exploited metal ion coordination.³ Another important class of fluorescent sensors designed in terms of PET has used reversible covalent bonding involving saccharides,⁴ even though the detailed mechanism is under discussion.⁵ Another class of PET fluorescent reagents/chemodosimeters involves irreversible covalent bonding.⁶ PET-based designs also sense membrane potential,⁷ environmental polarity^{8,9} and enzymes.¹⁰ We now demonstrate that rather specific receptor proteins and non-specific transport proteins⁹ can be exploited within the fluorescent PET sensing design, as exemplified by avidin and bovine serum albumin (BSA) respectively.

Since the avidin-biotin interaction is the basis of several technologies,¹¹ and since biotin enters a β -barrel of the protein to isolate the entrant from bulk water,¹² this protein-ligand interaction was attractive to apply the fluorescent PET sensor/switch design. Though the dimethoxybenzylanthracene unit cannot enter the β -barrel, its location in the water adjacent to the protein surface would be rather apolar, as evidenced by reduced H⁺ densities in related locations in water-organic interfaces for instance.¹³ We and others^{8,9} had demonstrated that apolar environments can cause strong switching 'on' of fluorescence by retarding the competing charge-separating PET process. Fine examples of selective 'off-on' fluorescent biotin-based sensors have been designed for avidin on the basis of polarity-sensitive ICT (internal charge transfer) excited states (one of which involves a separate PET process involving a thiol reaction),¹⁴ torsionally-restricted thiazole orange derivatives¹⁵ and disaggregation.¹⁶ There is a pseudointramolecular PET pair which dissociates upon engulfment of one partner by avidin so that the PET process ceases.¹⁷ A carbon nanotube is essential for this nanotechnology application and a small-molecule fluorophore with a tiny π -surface cannot be expected to undergo the necessary adsorption process. This is quite different from our case where the small-molecule PET pair is covalently linked and the PET rate is retarded by reducing the polarity of the environment by avidin since several PET pairs, e.g. anthracene-amine, anthracene-alkoxybenzene, anthracene-benzocrown ether, BODIPY-benzocrown ether, azaBODIPY-amine, have been shown to have polarity-dependent fluorescence.^{8,9} Though noncovalently-assembled PET and related systems^{17,18} have advantages of easier synthesis and combinatorial options, they are prone to the crucial weakness of disassembling in uncontrolled multicompartamental nanoenvironments (e.g. cells) and incorporating the components differentially into various compartments so that the fluorescence signal becomes indecipherable. In contrast, covalently linked PET systems¹⁹ have a history of successful operation in complex environments, and offer good prospects for cases like **1**.

PET between suitable anthracenes and *o*-dialkoxybenzenes is known in intermolecular situations²⁰ and has been exploited in several intramolecular contexts.²¹⁻²³ Sensor **1** arose from such thinking. It was synthesized from **2**²³ by reaction with **4**, the product of biotin coupled with N,N-diethylethylenediamine (DCC, see ESI).

It is clear from Fig. 1 that the fluorescence of **1** in water switches 'on' substantially upon encountering avidin at sub- μ M concentrations, while preserving other parameters of the spectrum as is usually found in fluorescent PET systems.²⁴ Similar concentrations of concanavalin A and BSA elicit smaller responses (Fig. 2). Derivative **3**, which lacks the biotin moiety, produces a negligible fluorescence response from avidin under similar conditions (Fig. 2). These control experiments suggest that the hydrophobicity of dimethoxybenzylanthracene moiety of **1** or **3** is not sufficient to cause a significant degree of non-specific association with apolar patches on the avidin surface. A further deduction from these experiments is that any non-specific protein-induced disaggregation is not significantly responsible for the avidin-induced fluorescence switching 'on', a point relevant to the role of disaggregation in the fluorescence enhancements found in sensors for sugars based on PET designs.^{4,5}

The log β value of 5.1 measured for the interaction of **1** with avidin is in the range of those found for various biotin derivatives. In contrast, the massive log β value of 15 found when underivatized biotin is bound inside the β -barrel is due to a peptide loop closing off the ligand for a tight fit.^{11,12} As a step towards demonstrating the generality of our approach, we find that **6**, which employs the anthracene-benzocrown ether PET pair, produces a fluorescence intensity enhancement factor of 6.1 when presented with 20 μ M avidin (see ESI, section S2 and Fig. 4a).

The structurally simpler **3** is subjected to a detailed study of its polarity-sensitive fluorescence. It was prepared by a procedure analogous to that of **1** (see ESI). The solvent-dependent quantum yield (ϕ_F) data are reasonably fitted to theory^{8,25} (Fig. 3 inset). This is consistent with a polarity-dependent process, such as a charge-separating PET pathway, being competitive against fluorescence emission for deactivation of the excited state.

The fluorescence response of **3** to the transporter protein BSA is shown in Figs. S1 and 3. The switching 'on' of fluorescence is attributed to retardation of PET by binding of **3** to a site in BSA. In contrast, previous fluorescence 'off-on' sensors for BSA are dominated by TICT (twisted intramolecular charge transfer) excited states,²⁶ except for a solitary example which is based on a fluorescent PET sensing design.⁹ **3** appears to bind non-selectively but similarly to the warfarin- and ibuprofen-binding sites of BSA according to drug-induced fluorescence quenching factors of 1.13 and 1.23 respectively (140 μ M drug). Further support for the generality of our approach is found in benzocrown ether **7**, which responds to 1.2 mM BSA with a FE value of 7.5 (see ESI, section S2 and Fig. 4b). This FE value and that of **6** due to Avidin are lower than those seen for the dimethoxybenzene versions **1** and **3** because the former cases are less hydrophobic and place their PET pair at an average position further from the protein surface where the polarity is higher, and hence PET is faster. Similar hydrophobic tuning of probe positions near membranes are known.¹³ Sensors **6** and **7** also show the extendability of our approach since they could allow measurement of metal ions in nanospaces adjacent to protein surfaces (as done in membrane-bounded nanospaces²²) near the active site. These double-input systems²⁷ share conceptual similarities with photoaffinity labels.²⁸ Na⁺ binding in the benzocrown causes further suppression of PET³ but the reduced hydrophobicity shifts the PET pair to a position of higher polarity¹³ which accelerates PET.⁹ The net effect on fluorescence depends on the nature of the protein (Fig. 4) and the sidechains of the amino acid residues neighbouring the charged units of the sensors.

We conclude that the fluorescent PET sensing/switching approach is a design platform for targeting proteins as illustrated by **1** and **6** for avidin and by **3** and **7** for BSA. Detailed evaluation of the mechanism will require laser photolysis studies to observe the presence (or not) of radical ion intermediates²⁹ which are outside the scope of this work. Nevertheless, using a ligand to carry a covalently linked fluorescent PET pair into a protein location extends the reach of fluorescent PET sensing/switching systems.

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Notes and references

‡ In memoriam Professor Tony McKervey

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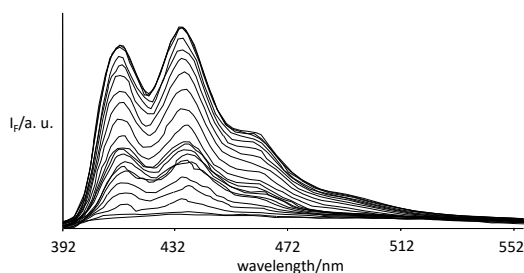
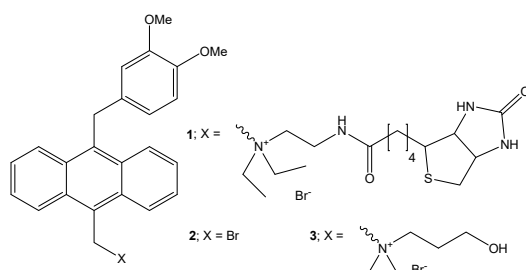


Figure 1. Fluorescence emission spectra for 10^{-5} M **1** (excited at 380 nm) in aqueous phosphate buffer (pH 7.2, 0.1 M) solution in the presence of avidin at 0.0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, 3.0, 4.2, 6.3, 8.4, 10.0, 13.0, 15.0, 17.0, 19.0, 23.0 and 25.0 μ M concentrations (in order of increasing intensity at 436 nm).



Scheme 1. Structures of sensors **1** and **3** and intermediate **2**.

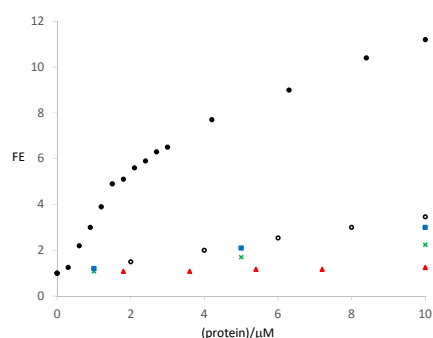


Figure 2. Protein-induced fluorescence intensity (I_F) enhancements (FE) as a function of protein concentration, according to the conditions given in Figure 1; **1**-avidin (filled circles), **1**-concanavalin A (open circles), **1**-BSA (blue squares), **3**-BSA (green crosses) and **3**-avidin (red triangles).

Table 1. Fluorescence and binding parameters for sensor-protein pairs.^a

Sensor	Protein	FE_{lim}^b	$FE_{10 \mu M}^c$	ϕ_F^d	$\log \beta^e$
1	avidin	15.1	11.2	0.094	5.1
1	con A	– ^f	3.5	– ^g	– ^g
1	BSA	– ^f	3.0	– ^g	– ^g
3	avidin	– ^f	1.25	– ^g	– ^g
3	BSA	16.0	2.2	0.068	4.2

a. Under conditions given in Fig. 1. b. limiting intensity is observable by maximization and levelling-off of the intensity as the concentration of the protein is increased as far as practicable. ϕ_F = 0.0063 for **1** and 0.0043 for **3**. c. at 10 μ M protein. d. fluorescence quantum yield (reference: 9,10-diphenylanthracene in aerated ethanol).³⁰ e. obtained from equation, $\log[(I_{Fmax} - I_F)/(I_F - I_{Fmin})] = pSensor + \log \beta$,³¹ by varying the concentration of sensor while holding the concentrations of avidin and BSA constant at 20 μ M and 200 μ M respectively. This necessitated the use of front-face observation at higher sensor

concentrations. f. limiting intensity not achieved within experimental range. g. not calculated since limiting intensity is not achieved.

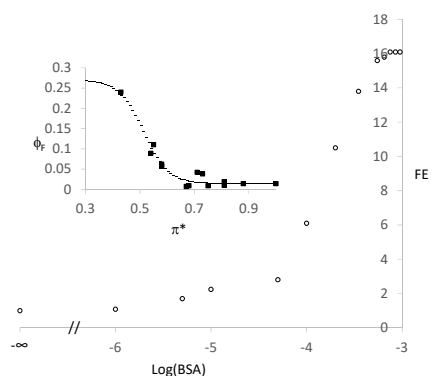


Figure 3. Fluorescence intensity enhancements (FE) of **3** induced by various concentrations of BSA (M units), according to the conditions given in Fig. 1. Inset: Fluorescence quantum yield (ϕ_F) of **3** as a function of solvent dipolarity (π^*). Experimental data (squares, see ESI) and data calculated (dashes) according to equation, $\log[(\phi_{Fmax} - \phi_F)/(\phi_F - \phi_{Fmin})] = B\pi^* + A$, where π^* is the Kamlet-Taft dipolarity,²⁵ and A and B are constants, with $\phi_{Fmax} = 0.27$, $\phi_{Fmin} = 0.014$, $B = 9.6$ and $A = -4.9$ for best fit to the experimental data.

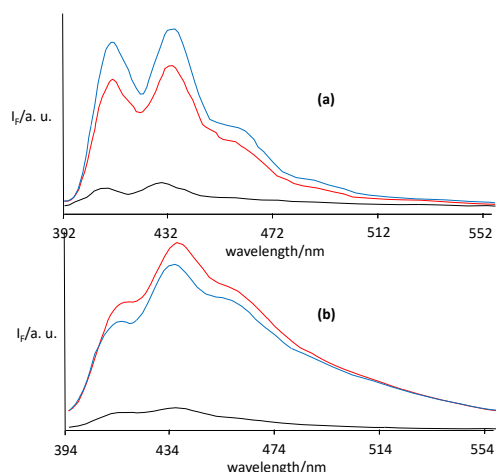
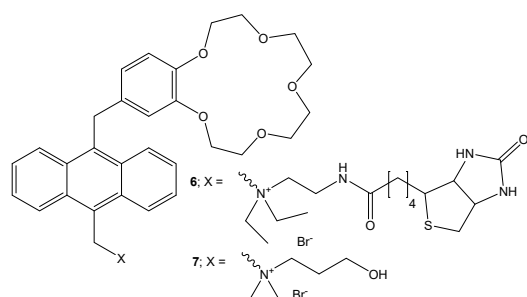


Figure 4. Structures of sensors **6** and **7** and their fluorescence spectra (a and b respectively) alone (black), with added 20 μ M avidin or 1.2 mM BSA (blue) and with added 1 M NaCl (red).

